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## Parasitism and Sex Ratio of the Bedeguar Gall Wasp *Diplolepis rosae* (L.) (Hymenoptera: Cynipidae) in Sicily (Italy)

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**Abstract.**—The *Diplolepis rosae* gall community is analysed in Sicily (Italy), based on collections totalling 82 galls from 12 sites from which 1,026 adult insects were obtained. The gall wasp exits in March–June from galls induced the previous year. On average 5.6 *D. rosae* individuals per gall were obtained, corresponding to 44.8% of all the emerged insects. We obtained 4.3% of *D. rosae* males overall, the highest figure found till now for the cynipid overall in Europe, where male *D. rosae* are usually rarer, and the first record of them for the Mediterranean area. No inquilines were found, and, consequently, none of their specific parasitoids. However, *Eurytoma rosae*, generally considered as a specific parasitoid of the inquiline *Periclistus brandtii*, was obtained, together with the polyphagous *Exeristes roborator*. In all, seven parasitoid species emerged from the galls: four of them, *Orthopelma mediator*, *Torymus bedeguaris*, *Exeristes roborator*, and *Eupelmus urozonus*, start to emerge together with *D. rosae*, while *Glyphomerus stigma*, *Pteromalus bedeguaris*, and *E. rosae*, have their maximum peaks later in the year. All the cited species, except for *E. roborator*, showed a second peak of emergence in September, when *D. rosae* is absent. Parasitization ranged from 12.5 to 100%, reaching more than 70% in 66.6% of the samples, but it was rather low (30.5%) when males were present, even though there was no overall correlation between parasitization and *D. rosae* sex ratio. Statistical analysis showed however that all the parasitoid species (except for males of *T. bedeguaris* and *P. bedeguaris*) are longer (which we take to signify larger) than *D. rosae* males, and neither the size nor the sex ratio of parasitoids differed statistically depending on the presence of male *D. rosae*.

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*Diplolepis rosae* (Linnaeus, 1758) is a palaearctic species, introduced in the nearctic region (Shorthouse and Ritchie 1984). It is widespread in peninsular Italy (Pagliano 1995), from where it has been known since at least 1600 (Tuscany: Pagliano et al. 1997), and in Sicily (De Stefani Perez 1887, as *Rhodites rosae* L.). Its multilocular galls have been recorded on 18 species of *Rosa* in Italy (De Stefani Perez 1887, 1902, 1905, Pellizzari Scaltriti 1988, Pagliano et al. 1997), and on 32 species in Europe and the Mediterranean basin (Houard 1908, Nieves-Aldrey 2001), though the gall wasp seems to prefer those species that are taxonomically allied in the Section Caninae (Randolph 2005), and particularly *R. canina* L. (Schröder 1967). Although *D. rosae* reproduces itself by amphitokous parthenogenesis (Beauvisage 1883, Callan 1940), males are repro-

ductively inactive (Stille and Dävring 1980) and of spasmodic occurrence probably due to female infection with *Wolbachia* bacteria, which is also common in other *Diplolepis* species (Plantard et al. 1998, 1999). Larvae grow inside the galls and overwinter in diapause as prepupae; they pupate the following spring and emerge soon after (Schröder 1967). *Diplolepis rosae* galls host a large insect community that has been extensively studied in many countries (Randolph 2005, and references therein). Considering only Hymenoptera parasitoids, Noyes (2003) lists 29 species of Chalcidoidea, and numbers increase when parasitoids belonging to other superfamilies are included (Fulmek 1968). However, several of the names listed by Fulmek (1968) are invalid or misidentifications or probably erroneously associated with *D.*



Table 1. Differences in the number of males and in the percentage of parasitization in different groups of gall samples.

	Total number of <i>Diplolepis rosae</i> emerging	Average number of <i>D. rosae</i> emerging per gall	Sex ratio (No. males/No. females)	% of <i>D. rosae</i> males as total of emerged insects	Average parasitization
Sicilian galls collected from 1992 to 2005 (n = 76)	394	5.18 (min.: 0; max.: 92)	0	0	57.6% (min.: 12.5%; max.: 100%)
Sicilian galls collected in 1965 (n = 6)	66	11	0.43	21	30.5%
Hungarian galls collected in 2001 (n = 2)	42	21	0.31	15.6	34.3%

*rosae* (Askew pers. comm.). Despite this deep knowledge of biology and ecology of the bedeguar gall wasp all over Europe, little or nothing is known about it in Italy (cf. Randolph 2005). The aim of this study is to rectify that situation, with special emphasis on aspects needing further investigation (*D. rosae* sex-ratio and parasitism), as suggested by Randolph (2005).

#### MATERIALS AND METHODS

A total of 82 galls were collected in the following localities in Sicily (Italy) (in brackets the number of galls per sample): Madonie Mts. (Palermo), 13.IV.65 (6); Madonie, Piano Pomo 2.II.96 (1); Madonie, Piano Zucchi 18.II.96 (1); Madonie, Piano Cervi 30.V.96 (1), 31.V.01 (1); Madonie, Fosso Canna 6.VI.99 (1); Madonie, Castelbuono 13.X.96 (1); Madonie, loc. Vicaretto 27.X.96 (10); Ficuzza (Palermo) 16.III.97 (5), 7.IX.97 (9), 7.II.99 (3), 28.III.99 (1), 27.VI.99 (2), 19.III.00 (8), 6.II.05 (2); Contessa Entellina, loc. S. Maria del Bosco (Palermo) XI.92 (1); Bivona (Agrigento) 29.X.96 (16); Nebrodi Mts. (Messina), Biviere di Cesarò 2.XI.96 (1), 17.X.99 (2); Pergusa (Enna), 13.XI.04 (10). One additional sample was collected in Hungary (Fertő-Hanság National Park, Köszeg, 16.V.01, 2 galls). Galls of *D. rosae* examined during this study were collected on *Rosa canina* L., *Rosa sempervirens* L. and *Rosa* sp. Galls were placed separately in single small cages, at room temperature in the Palermo laboratory. They were kept for at least one year

after the collecting date. In this way different emergence dates could be recorded for both gall inducers and parasitoids. Each emerged individual was counted, mounted and labelled. Parasitoid identification was carried out by M.C. Rizzo, if not differently reported in the Acknowledgements. Total body length, from the apex of the head to the tip of the abdomen, of all *D. rosae* adults and 30 specimens (males and females) of each parasitoid species was measured with the aid of a binocular microscope. Parasitoid length does not include the exerted ovipositor sheaths. Statistics were performed in STATISTICA (StatSoft 2003).

#### RESULTS AND DISCUSSION

*Sex ratio and phenology of Diplolepis rosae.*—A total of 460 individuals of *D. rosae* was obtained from the 82 galls examined, corresponding on average to 5.6 cynipids per gall and to 44.8% of all the emerged insects. However, while no males of *D. rosae* emerged from the 76 galls of 1992–2005, 21% males emerged from those collected in 1965 (Table 1). Also, in the latter sample, the average number of emerged *D. rosae* per gall was relatively higher (11 versus 5.18 of the other samples), while the percentage parasitization was somewhat lower than in the other samples on average (30.5 versus 57.6%) (Table 1). Table 1 also includes an occasional sample from Hungary, which also yielded a considerable number of males



Table 2. *Diplolepis rosae* sex ratio known until now (from Randolph 2005, except for the last five sets of data).

Country and Author	Total number of <i>D. rosae</i> individuals emerging	No. of males	% of males
USA: Kinsey (1920)	419	6	1.5
ENGLAND: Callan (1940)	6007	46	0.8
ENGLAND: Blair (1943)	104	2	1.96
ENGLAND: Niblett (1949)	601	1	0.17
NORTHERN ENGLAND: Askew (1960)	1264	51	4.2
DENMARK: Hoffmeyer (1925)	3425	137	4
GERMANY: Adler & Straton (1894)	671	7	1
GERMANY: Weidner (1956)	127	1	0.8
FRANCE: Hardouin (1943)	700	0	0
FRANCE, SPAIN, SWITZERLAND, AUSTRIA, SOUTH GERMANY: Schröder (1967)	2684	0	0
ENGLAND, ITALY, DENMARK, SWEDEN: Picard (1926)	>2000	4	<0.2
SOUTHERN SWEDEN: Nordlander (1973)	388	6	1.5
SPAIN: Nieves-Aldrey (1981)	249	0	0
SPAIN: Pujade Villar (1983)	49	0	0
SICILY (ITALY): this study (the whole Sicilian data set)	460	20	4.3
SICILY (ITALY): this study (Sicilian sample of 1965)	66	20	30.3
HUNGARY: this study (one sample, 2001)	42	10	23.8

(15.6% of the total insects); the number of cynipids emerged per gall (21) and percentage parasitization (34.3%) reflect those of the Sicilian sample of 1965 (cf. Table 1). Also considering Sicilian samples on the whole, *D. rosae* male percentage obtained during this study is among the highest reported in the literature (4.3%), and it is very much higher than the value given by Picard (1926) for Italy and other countries cumulatively (0.2%) (Table 2). High male figures for single samples, similar to those recorded by us (Sicilian sample of 1965: 30.3% of the total number of cynipids; Hungarian sample of 2001: 23.8%; cf. Table 2) are however reported by other authors (38%: Walsh 1924, 13%: Askew's unpublished data for a sample from Yorkshire (northern England), in Randolph 2005), even if never in the Mediterranean area. Askew (1960) suggested the presence of a decreasing latitudinal gradient (North>South) to explain the distribution of males of *D. rosae* in northern Europe, and data previously collected in southern Europe (reporting the complete absence of males) seemed to confirm his hypothesis (Nieves-Aldrey 1981, Pujade Villar 1983).

Our results, however, lead us to reconsider the existence of a latitudinal gradient; alternatively, we suppose that emergence of *D. rosae* males could depend on the combined effects of environmental and biological factors (such as female infection with *Wolbachia* bacteria and its spatial and temporal diffusion), as other authors have already hypothesized (Shorthouse and Ritchie 1984, and references therein, Plantard et al. 1998, 1999).

The cynipid phenology recorded during this study in Sicily matches what is already known for the species in North and central Europe (Callan 1940, Schröder 1967, Stille and Dävring 1980, Randolph 2005), even if its cycle starts earlier, probably because of the warmer weather: the majority of *D. rosae* adults exited galls in March (Fig. 1), with a few individuals continuing to appear till the end of the spring as recorded also by Schröder (1967) in central Europe. Male *D. rosae* of the 1965 Sicilian sample were obtained in May and June, at the same time as females, in contrast to the finding of Callan (1940), who recorded male appearance distinctly earlier than that of females. During this study neither the



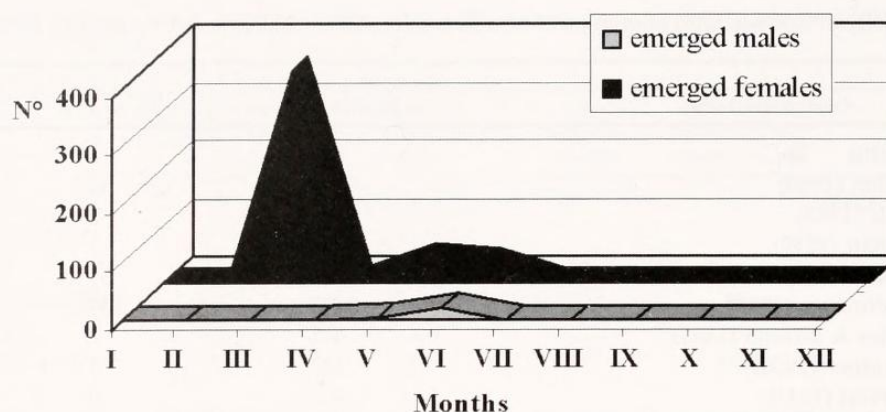


Fig. 1. Seasonal appearance of adults of *D. rosae* in Sicily.

inquiline *Periclistus brandtii* (Ratzeburg) nor its parasitoids were found. Although sometimes a common inhabitant of *D. rosae* galls, this species has a variable distribution in central and northern Europe and seems rarer in Mediterranean countries (Nieves-Aldrey 1981, Pujade Villar 1983, Randolph 2005). In Italy it is known only from the north (Pagliano 1995). No other inquiline species has been reared during this study.

**Parasitoid complex.**—Although a large number of parasitic wasps is associated with the bedeguar gall wasp, a typical list of parasitoids comprises about a dozen species (Schröder 1967, Askew 1984, Randolph 2005). During this study a total of 566 Hymenoptera, mainly belonging to the most common *D. rosae* parasitoid species, was obtained from the Sicilian galls as follows: 374 *Orthopelma mediator* (Thunberg) (= *O. luteolator* Gravenhorst) (Ichneumonidae), 98 *Torymus bedeguaris* (Linnaeus) (Torymidae), 52 *Glyphomerus stigma* (Fabricius) (Torymidae), 23 *Pteromalus bedeguaris* (Thomson) (= *Habrocytus bedeguaris* Thomson) (Pteromalidae), 12 *Exeristes roborator* (Fabricius) (Ichneumonidae), 5 *Eurytoma rosae* Nees (Eurytomidae), and 2 *Eupelmus urozonus* Dalman (Eupelmidae). They represented 55.2% of all insects and their relative abundance is reported in Fig. 2.

*Orthopelma mediator* is an endophagous species, largely known as a widespread

parasitoid of *D. rosae* (Askew 1960, Nieves-Aldrey 1981, Redfern and Askew 1992, Randolph 2005, and references therein), previously unrecorded from Sicily (Scaramozzino 1995, Noyes 2003). It was known as a parasitoid of *D. rosae* in Italy as early as 1600, since an unidentified Ichneumonidae, corresponding to it, is portrayed by Redi in an unpublished plate on *D. rosae* galls collected in Tuscany (Pagliano et al. 1997). In this study it was the most common (88.9% of the samples) and abundant parasitoid (66.1% of all parasitoids) (Fig. 2). *O. mediator* emerged from March onwards (Fig. 3), so that its phenology matches that of the gall inducer, as Nieves-Aldrey (1981) already observed in Spain, except in autumn when there was a late peak of emergences.

*Torymus bedeguaris* is a holarctic species (Grissell 1995, Noyes 2003), already recorded in Italy (De Stefani Perez 1905, Pagliano 1995, Pagliano and Navone 1995, Noyes 2003), mainly known as an ectoparasitoid of cynipid gall wasps belonging to the genus *Diplolepis* or of their inquelines (De Stefani Perez 1905, Askew 1960, Nieves-Aldrey 1981, Noyes 2003); it is also occasionally reported attacking *O. mediator* (Askew 1960, Schröder 1967). In Sicily *T. bedeguaris* represented 17.3% of all emerged parasitoids and its phenology overlaps that of *D. rosae* and *O. mediator* (cf. Figs 2 and 3). This trend is similar to that recorded by Nieves-Aldrey (1981) in Spain.



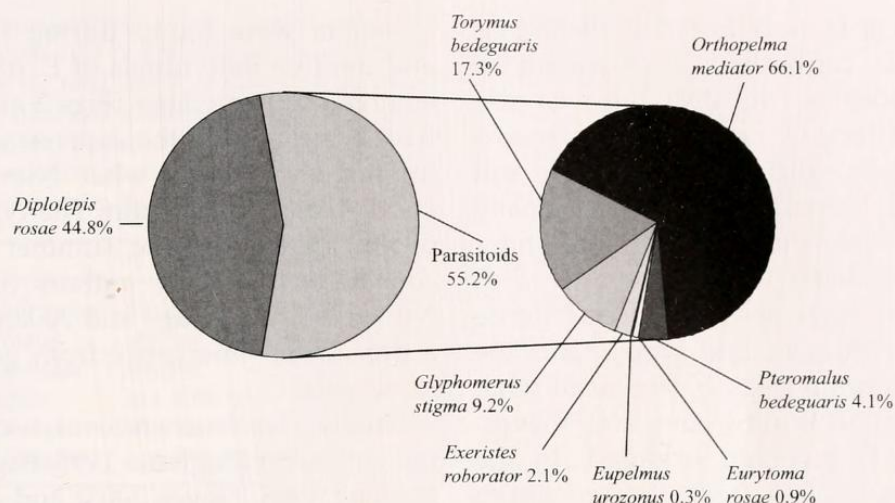


Fig. 2. Relative abundance of parasitoids of *D. rosae* in Sicily.

*Glyphomerus stigma* is another holarctic species typically associated with the genus *Diplolepis* (Noyes 2003), mainly known as an ectoparasitoid of *D. rosae* and its inquiline (Blair 1943, Askew 1960, Nieves-Aldrey 1981), and occasionally as a hyperparasitoid of *E. rosae* (Redfern and Askew 1992). This is the first record for Sicily, although the species was previously known from peninsular Italy (Pagliano 1995, Pagliano and Navone 1995, Noyes 2003). Nieves-Aldrey (1981) reported it as the second most abundant parasitoid in

Spain; in Sicily it reached only 9.2% of all parasitoids (Fig. 2). It appeared later in the year than the inducers and the previous parasitoid species (Fig. 3), which agrees with the findings of Nieves-Aldrey (1981) in Spain.

*Pteromalus bedeguaris* is one of the two species of Pteromalidae typically associated with the galls of *D. rosae*. It is a holarctic species, hitherto unknown in Italy (Pagliano 1995, Pagliano and Navone 1995, Noyes 2003), although De Stefani Perez (1905) listed many other Pteromalidae as

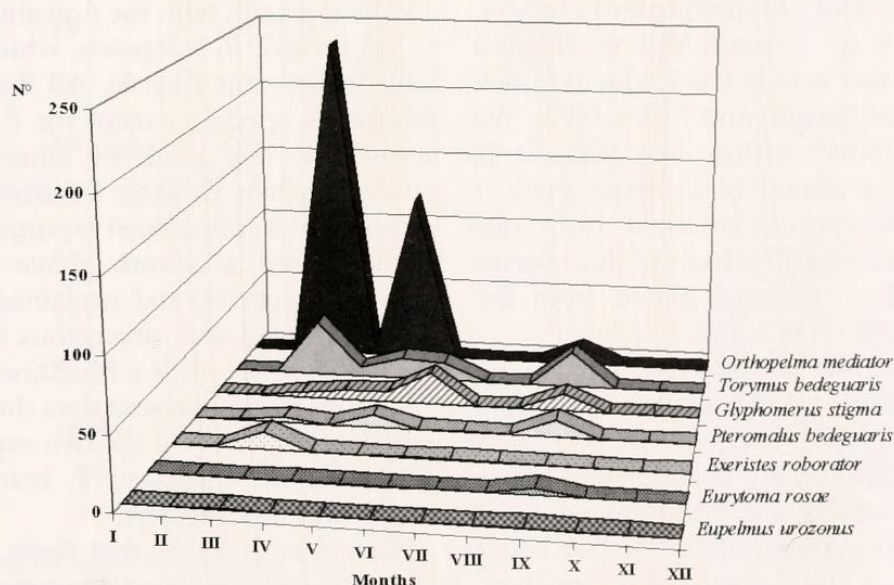


Fig. 3. Seasonal appearance of adults of parasitoids of *D. rosae* in Sicily.



parasitoids of *D. rosae* in Sicily. *Pteromalus bedeguaris* is considered a parasitoid of several species of *Diplolepis*, but it is also known to attack *O. mediator*, *T. bedeguaris* and *G. stigma* (Blair 1943, Redfern and Askew 1992, Noyes 2003), and occasionally parasitizes *Periclistus brandtii* (Nordlander 1973, Noyes 2003). The phenology of *P. bedeguaris* in Sicily shows two emergence periods, the first in late spring and the second in autumn (Fig. 3), similar to what Nordlander (1973) in Sweden and Nieves-Aldrey (1981) in Spain recorded. In the Sicilian samples, *P. bedeguaris* amounted to 4.1% of all emerged parasitoids (Fig. 2), while 22 individuals of this species (100% of all parasitoids) emerged from the Hungarian sample in June. This species is recorded as the second most common parasitoid in many countries of central Europe (Randolph 2005, and references therein). The palaearctic *Caenacis inflexa* (Ratzeburg) (Pteromalidae) was not obtained from any sample, being a specific parasitoid of the inquiline *P. brandtii* (Callan 1944), which itself was absent from our samples.

*Exeristes roborator* (Fabricius) is a palaearctic species, already known in Italy (Scaramozzino 1995). It is a polyphagous ectophagous parasitoid of Lepidoptera, Coleoptera and Hymenoptera larvae, known only as a parasitoid of *Biorhiza pallida* (Olivier) among Cynipidae (Fulmek 1968, Constantineanu and Pisica 1970). We cannot definitely affirm here that it is a primary parasitoid of *D. rosae*, even if no inquiline species emerged from our samples. Twelve individuals of this species (2.1% of all parasitoids) exited from the galls in March (Figs 2 and 3).

*Eurytoma rosae* is another palaearctic species, already known in Italy (Pagliano and Navone 1995, Noyes 2003), associated with galls induced by *Diplolepis* and considered as usually a specific parasitoid of *P. brandtii*, less commonly of *D. rosae* (Blair 1945, Claridge and Askew 1960). However, neither *P. brandtii* nor its specific parasitoid

*C. inflexa* were found during this study, and the five individuals of *E. rosae*, which emerged in September (Figs 2 and 3), may have developed at the expense of *D. rosae*, in agreement with what Nieves-Aldrey (1981) recorded in Spain. The typical peak of emergences in late summer has been considered by many authors (Blair 1945, Niblett 1951, Claridge and Askew 1960) as a precocious emergence from galls of the same year.

Finally, *Eupelmus urozonus*, a cosmopolitan generalist (Pagliano 1995, Pagliano and Navone 1995, Noyes 2003) and occasional parasitoid of *D. rosae* (Schröder 1967, Noyes 2003) has been obtained during this study: two individuals of this bivoltine species (Askew 1961) emerged in March and September.

*Parasitism and parasitoid relationships with the gall inducer.*—Parasitization ranged from 12.5 to 100%, reaching more than 70% in 66.6% of the samples, in line with other authors who found the bedeguar gall wasp heavily parasitized (Schröder 1967, Nordlander 1973, Stille 1984). In Sicily, the *D. rosae* parasitoid complex comprises two groups of species; namely, a first group, including *O. mediator*, *T. bedeguaris*, and *E. roborator*, whose phenology overlaps and strictly follows that of the gall inducer, and a second group, with the prevailing species *G. stigma* and *P. bedeguaris*, which appears later in the year (Fig. 3). All the recorded parasitoid species, except for *E. roborator*, showed a late peak of emergences in autumn, when *D. rosae* is absent (Fig. 3). This peak has often been reported by other authors (see accounts above of single parasitoid species) and explained, depending on species, as a precocious emergence (i.e. for *E. rosae*) or as a bivoltine cycle (i.e. *E. urozonus*). Our present data do not allow us to decide which of the two explanations applies to *O. mediator*, *T. bedeguaris*, *G. stigma* and *P. bedeguaris*.

Moreover, it seems that there is a negative association between the presence of *D. rosae* males and parasitization (cf. Table 1),



Table 3. Summarized results for the one way ANOVA analysis (FD = 273;  $F = 49.67$ ;  $p < 0.001$ ) performed on the total length of male and female *D. rosae* and its parasitoids (small samples were excluded from analysis). *D. rosae* males are statistically shorter than all the parasitoid species, except for *T. bedeguaris* and *P. bedeguaris* males (in bold).

	<i>D. rosae</i> females	<i>D. rosae</i> males
<i>O. mediator</i> both sexes	longer*	longer
<i>T. bedeguaris</i> males	shorter	<b>not different</b>
<i>T. bedeguaris</i> females	longer*	longer
<i>G. stigma</i> females	not different	longer
<i>P. bedeguaris</i> males	shorter	<b>not different</b>
<i>P. bedeguaris</i> females	not different	longer

\* Even if statistically longer, *O. mediator* and *T. bedeguaris* females overlap the maximum size of *D. rosae* females (Fig. 4), only *O. mediator* males being clearly longer. Moreover the two parasitoid species are much more slender than the massive *D. rosae* females, the latter probably being yet a suitable food source for them.

even if no correlation came out between parasitization and *D. rosae* sex ratio ( $r = -0.3$ ;  $fd = 16$ ,  $p = 0.21$ ). Comparing total lengths of *D. rosae* adults (males and females) with total lengths of their commonest parasitoids through a one way ANOVA analysis, we found that *D. rosae* males are statistically much shorter than all

the tested parasitoid species, except for *T. bedeguaris* and *P. bedeguaris* males (FD = 273;  $F = 49.67$ ;  $p < 0.001$ ) (Table 3 and Fig. 4). Given similar shapes of the species concerned, we take "shorter" to be a good indication of "smaller". From the ANOVA analysis parasitoid size did not differ between samples with or without males of *D. rosae* (cf. Fig. 4), and neither did their sex ratio differ (Wilcoxon Test;  $Z = 0.36$ ;  $p = \text{n.s.}$ ;  $fd = 4$ ). However, our data are insufficient to ascertain whether *D. rosae* males represent an inadequate food resource for parasitoid development and/or whether galls which contain them are avoided by parasitoids. It would be interesting to compare parasitization percentages in other parts of Europe where good numbers of males of *D. rosae* are recorded.

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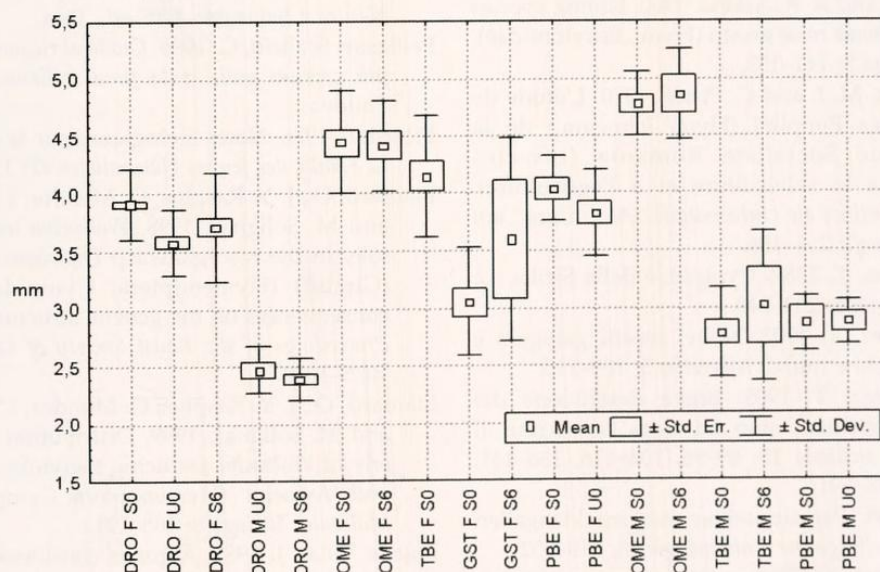


Fig. 4. Mean, s.d. and s.e. of the total length of male and female *D. rosae* and its parasitoids. (DRO = *D. rosae*; OME = *O. mediator*; TBE = *T. bedeguaris*; GST = *G. stigma*; PBE = *P. bedeguaris*; M = males; F = females; S0 = Sicilian samples 1992–2001; U0 = Hungarian sample 2001; S6 = Sicilian sample 1965).



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